

FILE COPY

MRID No.: 436491-07

**DATA EVALUATION RECORD**  
**S 72-1(A) -- ACUTE LC<sub>50</sub> TEST WITH A WARMWATER FISH**

1. **CHEMICAL:** Didecyldimethylammonium-carbonate (DDA Carbonate) **PC Code No.:** 069208  
2. **TEST MATERIAL:** DDA Carbonate **Purity:** 45.9%  
<sup>14</sup>C-DDA Chloride 96.9%

3. **CITATION**

**Author:** Maura K. Collins  
**Title:** Didecyldimethylammoniumcarbonate (DDA Carbonate) - Evaluation in a Static-Renewal Acute Toxicity Test with Bluegill Sunfish (*Lepomis macrochirus*)

**Study Completion Date:** June 20, 1994**Laboratory:** Springborn Laboratories, Inc., Wareham, MA**Sponsor:** Lonza Inc., Fair Lawn, NJ**Laboratory Report ID:** 94-5-5259**MRID No.:** 436491-07**DP Barcode:** D218362

- 4.
- REVIEWED BY:**
- Max A. Feken, M.S., Environmental Toxicologist, KBN Engineering and Applied Sciences, Inc.

**Signature:****Date:**

**APPROVED BY:** Mark Mossler, M.S., Toxicologist  
KBN Engineering and Applied Sciences, Inc.

**Signature:****Date:**

- 5.
- APPROVED BY:**

**Signature:****Date:**

- 6.
- STUDY PARAMETERS**

**Age or Size of Test Organism:** 29-41 mm**Definitive Test Duration:** 96 hours**Study Method:** Static-Renewal**Type of Concentrations:** Mean Measured

- 7.
- CONCLUSIONS:**
- This study is scientifically sound and fulfills the guideline requirements for an acute toxicity test using bluegill sunfish. An LC
- <sub>50</sub>
- value of 270 ppb ai would classify didecyldimethylammoniumcarbonate as highly toxic to bluegill sunfish. The NOEC was determined to be 98 ppb ai.



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**Results Synopsis**

LC<sub>50</sub>: 270 ppb ai  
 NOEC: 98 ppb ai

95% C.I.: 241-309 ppb ai  
 Probit Slope: N/A

**8. ADEQUACY OF THE STUDY**

**A. Classification:** Core

**B. Rationale:** N/A

**C. Repairability:** N/A

- 9. GUIDELINE DEVIATIONS** The pH of the 48-hour renewed solution was higher (8.4-8.7) than recommended (7.2 to 7.6). However, the pH returned to recommended levels by the end of the test (96-hour).

**10. SUBMISSION PURPOSE:****11. MATERIALS AND METHODS****A. Test Organisms**

Guideline Criteria	Reported Information
<b><u>Species</u></b> Preferred species is the bluegill sunfish ( <i>Lepomis macrochirus</i> )	<i>Lepomis macrochirus</i>
<b><u>Mean Weight</u></b> 0.5-5 g	0.59 g
<b><u>Mean Standard Length</u></b> Longest not > 2x shortest	Mean: 36 mm Range: 29-41 mm
<b><u>Supplier</u></b>	Bybrook Hatchery, Ashford, Connecticut
<b>All fish from same source?</b>	Yes
<b>All fish from the same year class?</b>	Not reported.

**B. Source/Acclimation**

Guideline Criteria	Reported Information
<b><u>Acclimation Period</u></b> Minimum 14 days	14 days.
<b>Wild caught organisms were quarantined for 7 days?</b>	N/A
<b>Were there signs of disease or injury?</b>	No
<b>If treated for disease, was there no sign of the disease remaining during the 48 hours prior to testing?</b>	N/A
<b><u>Feeding</u></b> No feeding during the study	No feeding 48 hours prior to testing or during test period.
<b><u>Pretest Mortality</u></b> No more than 3% mortality 48 hours prior to testing	0.4% mortality 48 hours prior to testing.

**C. Test System**

Guideline Criteria	Reported Information
<b><u>Source of dilution water</u></b> Soft reconstituted water or water from a natural source, not dechlorinated tap water	Soft reconstituted water with total hardness of 40 mg/L as CaCO <sub>3</sub> .
<b>Does water support test animals without observable signs of stress?</b>	Yes
<b><u>Water Temperature</u></b> 17°C or 22°C	20-23°C
<b><u>pH</u></b> Prefer 7.2 to 7.6	1 <sup>st</sup> half of test: 7.4 to 7.9 2 <sup>nd</sup> half of test: 7.5 to 8.7
<b><u>Dissolved Oxygen</u></b> Static: ≥ 60% during 1 <sup>st</sup> 48 hrs and ≥ 40% during 2 <sup>nd</sup> 48 hrs, flow-through: ≥ 60%	≥64% during 1 <sup>st</sup> 48 hrs and ≥59% during 2 <sup>nd</sup> 48 hrs
<b><u>Total Hardness</u></b> Prefer 40 to 48 mg/L as CaCO <sub>3</sub>	40 mg/L as CaCO <sub>3</sub>

Guideline Criteria	Reported Information
<b><u>Test Aquaria</u></b> 1. <b><u>Material:</u></b> Glass or stainless steel 2. <b><u>Size:</u></b> Volume of 19 L (5 gal) or 30 x 60 x 30 cm 3. <b><u>Fill volume:</u></b> 15-30 L of solution	1. Glass 2. 18.9 L 3. 15 liters
<b><u>Type of Dilution System</u></b> Must provide reproducible supply of toxicant	N/A
<b><u>Flow Rate</u></b> Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period	N/A
<b><u>Biomass Loading Rate</u></b> Static: $\leq 0.8$ g/L at $\leq 17^{\circ}\text{C}$ , $\leq 0.5$ g/L at $> 17^{\circ}\text{C}$ ; flow- through: $\leq 1$ g/L/day	0.39 g/L/day
<b><u>Photoperiod</u></b> 16 hours light, 8 hours dark	16 hours light, 8 hours dark
<b><u>Solvents</u></b> Not to exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-through tests	Solvent: None used Maximum conc.: N/A

#### D. Test Design

Guideline Criteria	Reported Information
<b><u>Range Finding Test</u></b> If $\text{LC}_{50} > 100$ mg/L with 30 fish, then no definitive test is required.	Range finding test concentrations: 100, 400, and 1000, 4000, and 10,000 $\mu\text{g}$ ai/L. No mortality or sublethal effects were noted at 100 $\mu\text{g}$ ai/L. Total mortality at $\geq 400$ $\mu\text{g}$ ai/L.

Guideline Criteria	Reported Information
<b><u>Nominal Concentrations of Definitive Test</u></b> Control & 5 treatment levels; dosage should be 60% of the next highest concentration; concentrations should be in a geometric series	Nominal test concentrations for definitive study: 53, 87, 140, 240, and 400 $\mu\text{g ai/L}$ . Dosage is 60% of next highest concentration. A dilution water control was also included.
<b><u>Number of Test Organisms</u></b> Minimum 10/level, may be divided among containers	20, 10 per replicate
<b>Test organisms randomly or impartially assigned to test vessels?</b>	Yes
<b>Biological observations made every 24 hours?</b>	Yes
<b><u>Water Parameter Measurements</u></b> 1. <u>Temperature</u> Measured constantly or, if water baths are used, every 6 hrs, may not vary $> 1^{\circ}\text{C}$ 2. <u>DO and pH</u> Measured at beginning of test and every 48 h in the high, medium, and low doses and in the control	1. Temperature measured constantly. 2. Yes; DO and pH measured for each treatment level and control at initiation and every 24 hours.
<b><u>Chemical Analysis</u></b> Needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used	Concentrations measured at 0-hour, 48-hour (time when solutions were renewed), and 96-hour. Mean measured concentrations were 58, 98, 140, 230, and 390 $\mu\text{g ai/L}$ .

## 12. REPORTED RESULTS

### A. General Results

Guideline Criteria	Reported Information
<b>Quality assurance and GLP compliance statements were included in the report?</b>	Yes

Guideline Criteria	Reported Information
<b>Recovery of Chemical</b>	97-110%
<b>Control Mortality</b> Not more than 10% control organisms may die or show abnormal behavior.	0%
<b>Raw data included?</b>	Yes
<b>Signs of toxicity (if any) were described?</b>	Yes

**Mortality**

Concentration (ppb)		Number of Fish	Cumulative Number Dead			
Nominal	Mean Measured		Hour of Study			
			24	48	72	96
Control	N/A	20	0	0	0	0
53	58	20	0	0	0	0
87	98	20	0	0	0	0
140	140	20	1	1	1	1
240	230	20	1	2	2	2
400	390	20	20	20	20	20

**Other Significant Results:** No signs of toxicity (sublethal effects) were observed in fish at the 98 and 58  $\mu\text{g ai/L}$  treatment levels or in the control group.

**B. Statistical Results**

Method: Binomial method

96-hr  $\text{LC}_{50}$ : 280 ppb ai

95% C.I.: 230-390 ppb ai

Probit Slope: N/A

NOEC: 98 ppb ai

**13. VERIFICATION OF STATISTICAL RESULTS**

Parameter	Result
Binomial Test LC <sub>50</sub> (C.I.)	283 (230-390) ppb ai
Moving Average Angle LC <sub>50</sub> (95% C.I.)	270 (241-309) ppb ai
Probit LC <sub>50</sub> (95% C.I.)	N/A
Probit Slope	Not determined.
NOEC	98 ppb ai

- 14. REVIEWER'S COMMENTS:** This study is scientifically sound and fulfills the guideline requirements for an acute toxicity test using bluegill sunfish. An LC<sub>50</sub> value of 270 ppb ai would classify didecylldimethylammoniumcarbonate as highly toxic to bluegill sunfish. The NOEC was determined to be 98 ppb ai. This study is classified as **Core**.

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
390	20	20	100	9.536742E-05
230	20	2	10	2.012253E-02
140	20	1	5	2.002716E-03
98	20	0	0	9.536742E-05
58	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 230 AND 390 CAN BE  
USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT  
CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL  
ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 282.8375

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
2	6.572952E-02	270.1171	240.9739	309.3684

NO CONVERGENCE IN 25 ITERATIONS. THE PROBIT METHOD  
PROBABLY CANNOT BE USED WITH THIS SET OF DATA.

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